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Y chromosome peculiarities and chromosomal G- and C-staining in *Crocidura shantungensis* Miller, 1901 (Soricomorpha: Soricidae)

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Abstract. Cytogenetical examinations of *Crocidura shantungensis* Miller, 1901 from small Young Island of South Korea and the mainland of Russian Far East (Vladivostok) were carried out and literature data concerning Tsushima Island of Japan and Cheju Island of Korea were considered. The chromosome sets of all investigated specimens are characterized by 2n = 40 and NFa = 46. Four pairs of biarmed autosomes, 15 pairs of acrocentrics and two sex chromosomes were identified applying G- and C-banding. The X chromosome is a large metacentric whereas the Y chromosome is a middle sized acrocentric element. A variation in the Y chromosome size in samples from the three islands is noticeable. Comparative analysis of G-banded chromosomes of *C. shantungensis* (our material) and *C. suaveolens* (Pallas, 1811) (literature data) shows a similarity between karyotypes of these two species.

Key words: *Crocidura shantungensis*, mammalian karyotype, chromosomal polymorphism, Y chromosome, G-banding, C-heterochromatin.

Introduction

White-toothed shrews of the genus *Crocidura* Wagler, 1832 are widely distributed in the Ethiopian, Palearctic, and Oriental regions (Hutterer, 1993; Wolsan, Hutterer, 1998). Approximately 8-10 species of crocidurine shrews are known from the East Asia (Ohdachi et al., 2004), while in NE China, Korea and Russian Far East only two species of this genus differing in body size are distributed: *Crocidura lasiura* Dobson, 1890 of a larger size and *Crocidura suaveolens* (Pallas, 1811) of a lesser size (Jones, Johnson, 1960). Recent studies of the mitochondrial cytochrome b gene sequence uncovered that *Crocidura shantungensis* inhabits Eastern part of Asia while typical *C. suaveolens* occurs in Europe (Ohdachi et al., 2004).

In view of the growing interest in crocidurine karyotypic (Biltueva et al., 2001; Motokawa et al., 2004) and molecular relationships (Iwasa et al., 2001; Motokawa et al., 2005), further information on the two above mentioned species is needed. Regarding *C. shantungensis*, descriptions of the chromosomal set (under the species name *C. suaveolens*) were presented from two islands located between southern parts of Korea and Japan: Cheju (locality 3 in Fig. 1) and Tsushima (locality 4). The same diploid and fundamental

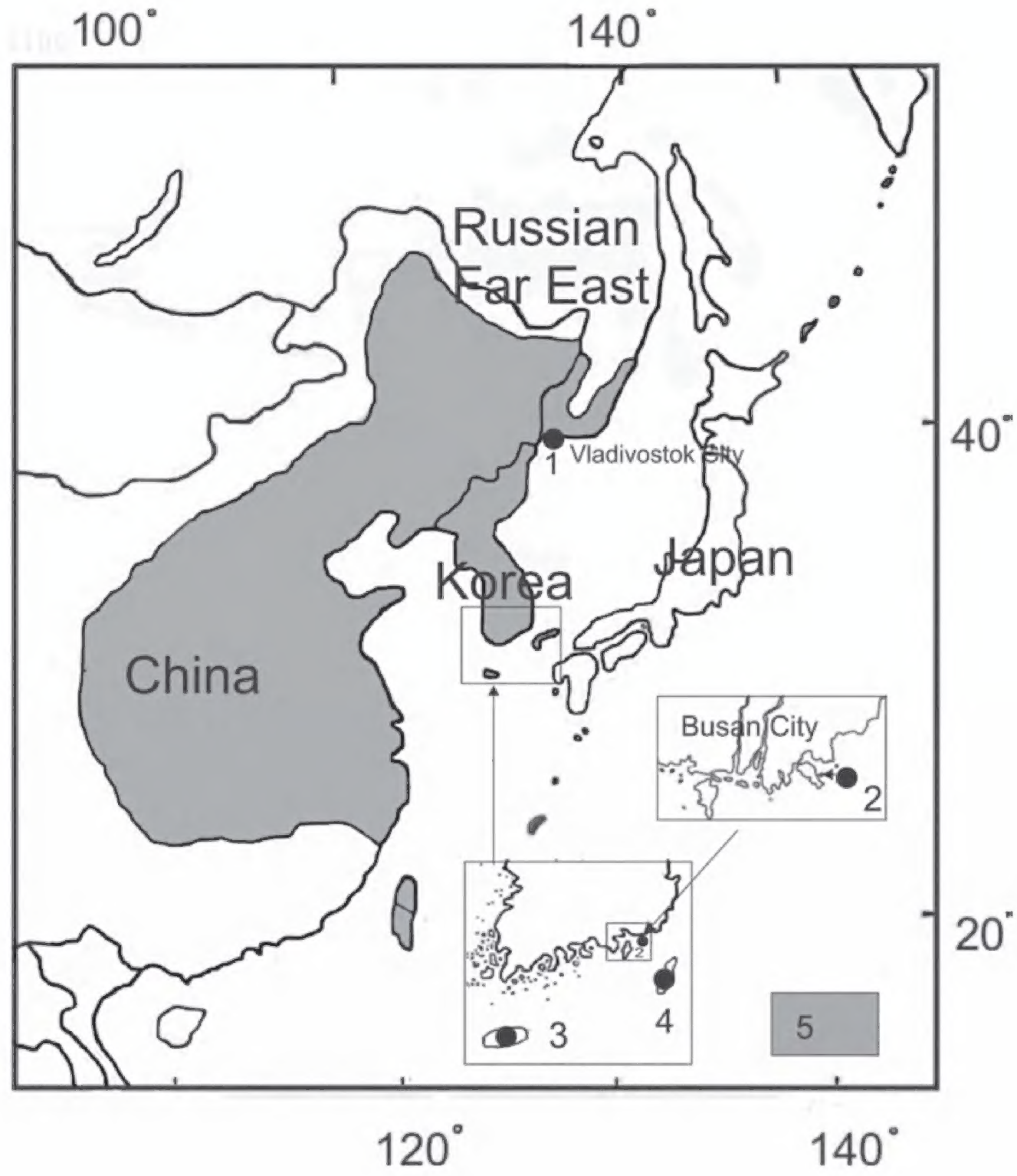

Fig. 1. Map showing the species range and known sampling sites of karyological investigation for *Crocidura shantungensis*: 1 – Vladivostok, Russian Far East; 2 – Young Island, S. Korea; 3 - Cheju Island, S. Korea; 4 - Tsushima Island, Japan; 5 – mainland distribution.

chromosome numbers 2n = 40 and NF = 50 were reported in both cases. On Tsushima Isl., a sample of 8 specimens displayed an autosomal Robertsonian heterozygote in one female (NF=39) and an acrocentric Y chromosome in two males studied (Tsuchiya, 1987). On Cheju Isl., a dot like Y chromosome was detected in a single captured male (Iwasa et al., 2001). Differential staining of chromosomes of *C. shantungensis* was still unknown.

In this communication we report chromosomal data on *C. shantungensis* from new findings in a Korean island as well as from the mainland coast in Russia. For the first time, the species karyotype has been studied applying methods of differential chromosome staining.

Materials and methods

Nine shrew individuals were obtained for karyological examination, including 1 male from Russia, Botanical Garden near Vladivostok (Fig. 1 - locality 1: 43°13'486" N/ 131°59'277" E) and 8 specimens (5 ♀♀, 3 ♂♂) from the Korean Young Island, a territory of the Maritime State University in Busan (locality 2: 35°04'35.6" N/ 129°05'35.6" E).

Standard chromosome preparations were made from bone marrow and spleen cells of each specimen (Ford, Hamerton, 1956). Conventional procedures of differential G- and C-staining (Seabright, 1971; Sumner, 1972) were applied.

Results and discussion

The chromosomal sets of all 9 animals studied revealed general similarity in 2n (40) and NFa (46) containing four bi-armed pairs, submeta - (SM), meta – (M), or subtelocentrics (ST), and 15 pairs of acrocentrics (A) among autosomes. The X chromosome was a large submetacentric, while the Y chromosome is an ordinary acrocentric (Fig. 2).

Differential chromosome staining (C- and G- banding) has been obtained only for chromosomes of *C. shantungensis* from Korea (Fig. 2, b-c). A G-banded karyogram was constructed to compare chromosomes of *C. shantungensis* (our data) and *C. suaveolens* from N Caucasus as presented by Graphodatsky et al. (1988). A general similarity between two 40-chromosome genomes at the level of their G-banding has been revealed (Fig. 2, c). The same G-banding patterns were reported for chromosomes of other two crocidurine species, *C. gueldenstaedtii* Pallas, 1811 and *C. sibirica* Dukelsky, 1930 (Biltueva et al., 2001).

The use of C-banding enabled a correct identification of the Y chromosome among the similar acrocentrics in the Korean specimens of *C. shantungensis*. It was completely heterochromatic, comparable in size to the long arm of X chromosome (Fig. 2, b). Small C-positive blocks are located in pericentromeric position of all chromosomes and also in the telomeric region of the X short arm. And also, C-band pattern resembles well that one reported for *C. suaveolens* (Graphodatsky et al., 1988).

The Y chromosome variation exists in *C. shantungensis* from different East Asian localities investigated. This odd male chromosome was found in 2 or 3 variants: a similar in size acrocentric (Young Island of Korea) or subtelocentric (Tsushima Island of Japan and a continental population in Russian Far East) and the sharply decreased tiny acrocentric reported for a male from the Korean Cheju Island (Fig. 2, d). Because only the acrocentric variant has been confirmed by C-band staining (Fig. 2, b), the recognition of the subtelocentric Y-chromosome remains questionable. As for the geographical variation in the Y chromosome size and shape, it is well known in the house shrew, *Suncus murinus* (Linnaeus, 1766) (Soricidae) from different islands to the east, southeast and southwest of Asian mainland (Yosida, 1982).

Phylogenetic analysis of molecular markers has shown a peculiar position of *C. shantungensis* from Cheju Isl. (Ohdachi et al., 2004). This insular population seems more divergent from the mainland samples, as was the case with shrews occupying other islands: Putjatin, Popov in the Russian Far East and Ullung in South Korea. Though the corresponding chromosome data were absent from the last sites, the materials concerned in our study might be interpreted as supporting the accumulation of genetic changes and, namely, in the Y chromosome due to the long time isolation of population on the Cheju Island.

Detailed investigations of *C. shantungensis* karyotypes from populations isolated on East

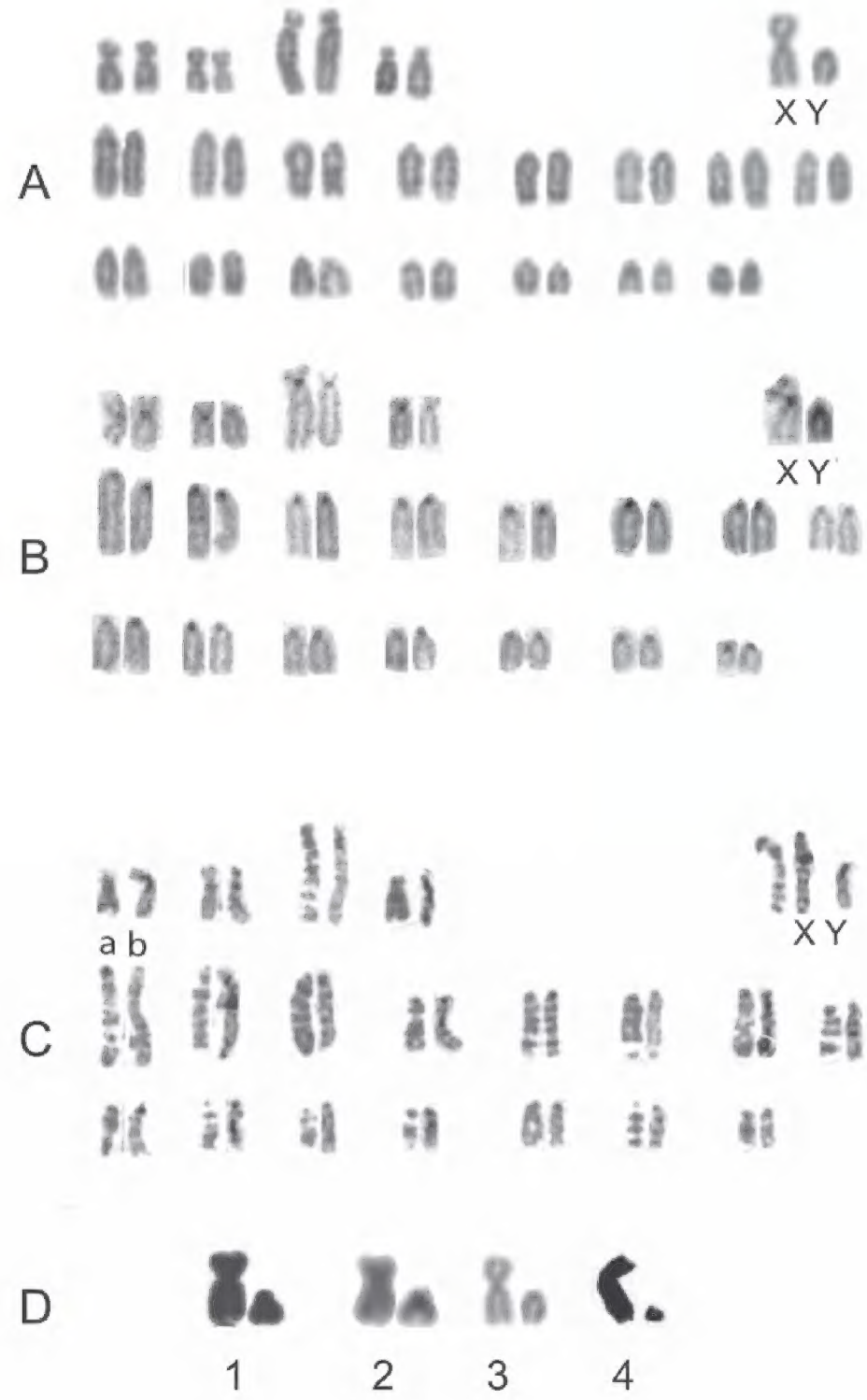

Fig. 2, a-d. Karyotypes and Y chromosome of *C. shantungensis*. **a** - routine stained chromosomes, and **b** - C-banded karyogram of a male from Young Isl. **c** - comparison of G-banded haploid complements of *C. suaveolens* (left chromosome in each pair, female, after Grafodatsky et al., 1988) and *C. shantungensis* (right chromosome in each pair, male, our data). **d** - male heterochromosomes of *C. shantungensis* (**1** - Young Isl. (our data), **2** - Vladivostok (our data), **3** - Tsushima Isl. (Tsuchiya, 1987), **4** - Cheju Isl. (Iwasa et al., 2001)).

Asia islands or distributed on the continent are needed to complete the evolutionary studies of the genomes of the total *Crocidura suaveolens* species group (Bannikova et al., 2006).

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References

Bannikova A.A., Lebedev V.S., Kramerov D.A., Zaitsev M.V. 2006. Phylogeny and systematics of the *Crocidura suaveolens* species group: corroboration and controversy between nuclear and mitochondrial DNA markers // *Mammalia*. 70: 106-119.

Biltueva L.S., Rogatcheva M.B., Perelman P.L., Borodin P.M., Oda S-I., Koyasu K., Harada M., Zima J., Graphodatsky A.S. 2001. Chromosomal phylogeny of certain shrews of the genera *Crocidura* and *Suncus* (Insectivora) // *J. Zool. Syst. Evol. Res.* 39: 69-76.

Ford C.E., Hamerton J.L. 1956. A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes // *Stain Technol.* 31: 247–251.

Graphodatsky A.S., Radjabli S.I., Sharshov A.V., Zaitsev M.V. 1988. Karyotypes of five *Crocidura* species of the USSR fauna // *Tsitologiya.* 30: 1247-1252. (In Russian).

Hutterer R. 1993. Order Insectivora, (pp. 69-130) // Wilson D.E., Reeder D.M. (Eds). Mammal species of the world: a taxonomic and geographic reference. Washington D.C. 1207 p.

Iwasa M.A., Ohdachi S.D., Han S–H., Oh H–S., Abe H., Suzuki H. 2001. Karyotype and RFLP of nuclear rDNA of *Crocidura* sp. on Cheju Island, South Korea (Mammalia, Insectivora) // *Mammalia.* 65: 451-459.

Jones J.K. Jr., Johnson D.H. 1960. Review of the insectivores of Korea // *Univ. Kansas Publ.* 9: 549-578.

Motokawa M., Harada M., Lin L-K. 2004. Variation in the Y chromosome of *Crocidura tadae kurodai* (Insectivora, Soricidae) // *Mammalian Biol.* 69: 273-276.

Motokawa M., Yu H-T., Harada M. 2005. Diversification of the white-toothed shrews of the genus *Crocidura* (Insectivora, Cricetidae) in East and Southeast Asia // *Mammal Study.* 30: 53-64.

Ohdachi S.D., Iwasa M.A., Nesterenko V.A., Abe H., Masuda R, Haberl W. 2004. Molecular phylogenetics of *Crocidura* shrews (Insectivora) in East and Central Asia // *J. Mammal.* 85: 396–403.

Seabright M.A. 1971. Rapid banding technique for human chromosomes // *Lancet.* 11: 971–972.

Sumner A.T. 1972. A simple technique for demonstrating centromeric heterochromatin // *Exp. Cell Res.* 75: 304–306.

Tsuchiya K. 1987. Cytogenetic and biochemical studies of Insectivora in Tsushima Island, (pp. 111-124) // *Nature of Tsushima*: Tsushima Nat. Res. Rep. Nagasaki Prefecture. (In Japanese).

Wolsan M., Hutterer R. 1998. A list of the living species of shrews, (pp. 425-448) // Wójcik J.M., Wolsan M. (Eds). *Evolution of shrews*. Białowieża. 548 p.

Yosida T.H. 1982. Cytogenetical studies on Insectivora. II. Geographical variation of chromosomes in the house shrew, *Suncus murinus* (Soricidae), in East, Southeast and Southwest Asia, with a note on the karyotype evolution and distribution // *Japan J. Genet.* 57: 101-111.

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